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Remarks:

•A request for correction of the sequences of SEQ ID N° 1-6 and 9 has been filed pursuant to Rule 139 EPC. A decision on the request will be taken during the proceedings before the Examining Division (Guidelines for Examination in the EPO, A-V, 3.).  
•Amended claims in accordance with Rule 137(2) EPC.

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(54) **Identification of the microVar strain of Ostreid herpesvirus 1 (OsHV-1)**

(57) The present invention concerns a method for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, which method comprises the steps of (i) determining the sequence of

the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus, and (ii) determining if said OsHV-1 virus corresponds to the OsHV-1  $\mu$ Var strain; and a pair of primers and a kit useful for such a method.

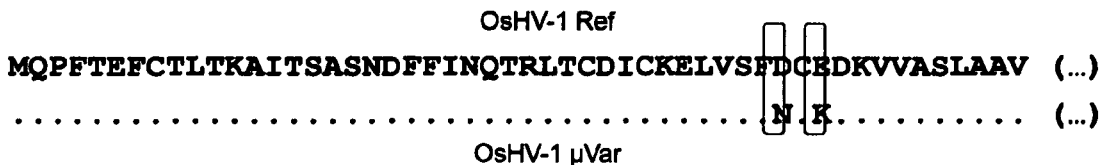


Figure 2

**Description**

**Field of the invention**

5 [0001] The present invention relates to mollusc infection by Herpes-like viral and is directed to oligonucleotides, methods and kits which are especially useful as an aid in the diagnosis of an infection with the  $\mu$ Var strain of Ostreid herpesvirus 1 (OsHV-1).

**Background of the invention**

10 [0002] Herpes-like viral infections have been reported in different bivalve mollusc species throughout the world. More specifically, Ostreid herpesvirus 1 (OsHV-1) is the only member of the Herpesviridae that has an invertebrate host and is associated with sporadic mortality in the Pacific oyster (*Crassostrea gigas*) and other bivalve species.

15 [0003] Following the characterisation and genome sequencing of ostreid herpesvirus 1 (OsHV-1), specific diagnostic tools have been developed based on conventional PCR techniques, in situ hybridisation and quantitative PCR.

[0004] In France, the year 2008 displayed high mortality rate ranging from 80% to 100% affecting only cupped oyster, *Crassostrea gigas* and mainly juvenile oysters from May to September.

[0005] According to devastating of losses, it appeared important to investigate if this high mortality rate could be associated to emerging or new pathogens.

20 [0006] Because of preliminary tests for herpesvirus detection showed around 75% positive batches; the question was whether it could be related to the emergence of a new genotype of Ostreid Herpesvirus type 1 (OsHV-1).

**Summary of the invention**

25 [0007] The present invention relates to a method for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, which method comprises the step of (i) determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus, and (ii) determining if said OsHV-1 virus corresponds to the OsHV-1  $\mu$ Var strain.

30 [0008] The present invention further relates to a pair of oligobucleotides useful for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, wherein said pair of oligobucleotides have the sequences GTG ATG GCT TTG GTC AAG GT (SEQ ID n°7) and CCC CGG GGA AAA AGT ATA AA (SEQ ID n°8).

[0009] Finally, the present invention relates to a kit for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain.

**Brief description of the drawings**

[0010]

The figure 1 shows the identified polymorphisms in C2/C6 fragment associated with increased mortality.

The figure 2 shows the ORF4 amino acids changes associated with OsHV-1  $\mu$ Var sequence.

The figure 3 shows identified polymorphisms in IA1-IA2 fragments also associated with OsHV-1  $\mu$ var sequence.

45 The figure 4 shows an electrophoresis gel of PCR products obtained with CF/CR primers realized on 2 OsHV-1 isolates and 2 *OsHV-1*  $\mu$ Var isolates. Only two genotype  $\mu$ Var (1a and 12) and two genotype reference OsHV-1 (3 and 4) are presented. C: positive control. M: DNA ladder.

**Detailed description**

50 [0011] The inventors have now identified a new OsHV-1 genotype or strain in 40% of studied spat batches from 2008 called OsHV-1  $\mu$ Var, which could be considered as an emerging viral genotype involved in oyster mortalities.

[0012] Specific polymorphisms of this genotype have been identified by the inventors, which polymorphisms are located in two targeted regions C and IA, with reference to OsHV-1 DNA.

55 [0013] These polymorphisms correspond as an example to a deletion in a microsatellite zone and to substitutions in coding zones of the ORF4 protein.

[0014] Finally, the inventors have identified a PCR primers pair enabling a simple and fast diagnosis of oyster OsHV-1  $\mu$ Var infection.

[0015] Consequently, the present invention relates to a method for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, which method comprises the step of:

5 i) determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus, and

ii) determining if said OsHV-1 virus corresponds to the OsHV-1  $\mu$ Var strain.

[0016] Said particular OsHV-1 virus is isolated or not from a biological sample.

10 [0017] As used herein, said biological sample can correspond to plankton, mollusc or seawater.

[0018] The step of determining if said OsHV-1 virus corresponds to a OsHV-1  $\mu$ Var strain in view of its sequence can be simply done by the skilled person in view of the polymorphisms identified by the inventors.

[0019] In fact, the inventors have established that the following nucleic acid polymorphisms are characteristic of the OsHV-1  $\mu$ Var strain as compared to the reference DNA of OsHV-1:

15 ● For SEQ ID n°1, at least an A instead of a G at position 151, a C instead of AA at position 223, an insertion of GC at position 249 instead of no nucleic acid, a G instead of C at position 253, an A instead of G at position 257, no nucleic acid instead of AC at position 306, G instead of ACTACTACTACTGA (SEQ ID n°3) at position 360, A instead of G at position 482, and/or a A instead of G at position 488.

20 ● For SEQ ID n°2, no nucleic acid instead of an A at position 110 and/or a T instead of a C at position 526.

[0020] Thus, the sequences SEQ ID n°4 and SEQ ID n°5 are characteristic of the C and IA regions respectively of the  $\mu$ Var C genotype.

25 [0021] It should be further noticed that, as a result of some of the previously disclosed nucleic acid polymorphisms, the following amino acid polymorphisms are characteristic of the OsHV-1  $\mu$ Var strain as compared to the reference DNA of OsHV-1:

30 ● For ORF4 (SEQ ID n°6), at least a N instead of a D at position 40 and/or a K instead of a E at position 42.

[0022] Advantageously, said determining step (ii) is done by identifying at least one of the previously disclosed polymorphisms.

[0023] In a preferred embodiment, said method comprises the step of determining the sequence of the C region (SEQ ID n°1) of the genomic DNA of said OsHV-1 virus.

35 [0024] In another preferred embodiment, said determining step (ii) is done by identifying the polymorphism corresponding to G instead of ACTACTACTACT6A (SEQ ID n°3) at position 360 of the C region (SEQ ID n°1) of the genomic DNA of said OsHV-1 virus.

[0025] In a still preferred embodiment, the method of the invention is a method for diagnosis an infection by an OsHV-1  $\mu$ Var strain in a mollusc, wherein said method further comprises a previous step of determining the presence or the absence of an OsHV-1 virus in a biological sample of said mollusc.

40 [0026] Thus, the biological sample is a mollusc, or a part thereof, and most preferably a soft tissue of said mollusc.

[0027] The step of determining the presence or the absence of an OsHV-1 virus in a biological sample of said mollusc can be done by well known methods such as those disclosed in PEPIN et al. (J VirolMethods, vol.149(2), p:269-76, 2008).

[0028] As used herein, a mollusc is a bivalve mollusc selected in the group comprising oysters, scallops and clams.

45 [0029] Preferably, said mollusc is an oyster and most preferably a cupped oyster such as *Crassostrea gigas*

[0030] The step (i) of determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus can be done directly by sequencing of these regions or indirectly by hybridization techniques such as DNA array or Southern blot, by enzymatic digestion techniques such as RFLP, by amplification techniques such as Polymerase Chain Reaction (PCR), Southern Blot, Northern Blot, or by enzymatic techniques such as PRA (PCR-restriction length polymorphism analysis).

50 [0031] Preferably, said determining step (i) is done by amplification techniques and most preferably by PCR.

[0032] Useful primers for said amplification techniques have a length of at least 12 nucleic acids, preferably of at least 15 nucleic acids and most preferably of at least 20 nucleic acids.

[0033] In a still preferred embodiment, the determining step (i) is done by PCR using the pair of primers CR (GTG ATG GCT TTG GTC AAG GT, SEQ ID n°7) and CF (CCC CGG GGA AAA AGT ATA AA, SEQ ID n°8).

55 [0034] Said pair of primers enables a simple and fast identification of a OsHV-1 virus corresponding to a  $\mu$ Var OsHV-1 strain by PCR, since samples comprising DNA of said strain result in a 158pb amplicon (SEQ ID n°9).

[0035] A second aspect of the invention concerns a pair of oligonucleotides useful for determining if a particular Ostreid

herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, wherein said pair of oligobucleotides have the sequences GTG ATG GCT TTG GTC AAG GT. (SEQ ID n°7) and (CCC CGG GGA AAA AGT ATA AA (SEQ ID n°8).

[0036] A third aspect of the invention concerns a kit for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, wherein said kit comprises:

- means for determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus.

[0037] As an example, such means can include primers for amplifying the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus, or antibodies recognizing the ORF4 protein specifically expressed by the OsHV-1  $\mu$ Var strain comprising at least a N instead of a D at position 40 and a K instead of a E at position 42 of ORF4 (SEQ ID n°6).

[0038] Advantageously, said means corresponds to the previously described pair of oligonucleotides.

[0039] The kit of the invention may comprise additional reagents useful for the extraction of nucleic acids from a tissue sample or for analyzing the nucleic acid extracted from a tissue sample such as polymerase chain reaction reagents. The kit may also comprise specific controls based on purified plasmidic DNA from clones containing insert C2/C6 fragments as targeted DNA matrix originating from reference or  $\mu$ Var genotype Oshv-1 isolates.

[0040] The following experiments are offered to illustrate embodiments of the invention and should not be viewed as limiting the scope of the invention.

## Examples

### Identification of a new genotype of OsHV-1 associated with increased Oyster mortality

[0041] DNA was extracted from Oyster soft tissue as disclosed in PEPIN *et al.* (abovementioned, 2008).

[0042] The used DNA corresponds to 24 batches from field mortality events >70% in 2008, 4 batches previously used in study of challenge in 2008 and to 49 DNA samples from several years and different countries.

[0043] OsHV-1 DNA were identified in said samples and regions of said OsHV-1 DNAs were sequenced.

[0044] The analysis of said sequences as compared with OsHV-1 reference DNA (AY509253, GenBank) revealed several polymorphisms in ORF4 and ORF43 regions.

[0045] The figure 1 shows the identified polymorphisms in C2/C6 fragment associated with increased mortality.

[0046] These nucleic acids polymorphisms result in ORF4 amino acids changes as shown in Figure 2.

[0047] The figure 3 shows identified polymorphisms in IA1-IA2 fragments also associated with increased mortality.

[0048] These polymorphisms are specific of the new OsHV-1 genotype (strain)  $\mu$ Var and their detections enable to diagnose an infection with this specific genotype.

### Identification of a new genotype of OsHV-1 associated with increased Oyster mortality

[0049] DNA was extracted from Oyster larvae infected with OsHV-1  $\mu$ Var genotype. DNA is then diluted to a concentration comprised between 10 and 500ng DNA/ $\mu$ l

[0050] As a positive control, OsHV-1 reference genomic DNA (Accession Number : AY 509253) was used, which corresponds to the one purified from oyster larvae infected in 1995 by OsHV-1 virus. The concentration of this reference OsHV-1 genomic DNA was about 2 pg/ $\mu$ l corresponding to 50.10<sup>3</sup> DNA copies/ $\mu$ l.

[0051] So as to identify OsHV-1  $\mu$ Var genotype, specific primers CR (GTG ATG GCT TTG GTC AAG GT, SEQ ID n°7) and CF (CCC CGG GGA AAA AGT ATA AA, SEQ ID n°8) have been designed.

[0052] The following melting on the basis of MIX RED GOLD STAR kit (EUROGENTEC) was prepared:

Compound	Volume
Buffer 10x	5 $\mu$ l
MgC1 <sub>2</sub> (25mM)	5 $\mu$ l
dNTP (2mM)	5 $\mu$ l
Primer CR (10 $\mu$ M)	1 $\mu$ l
Primer CF (10 $\mu$ M)	1 $\mu$ l
Taq RED (5U/ $\mu$ l)	0,5 $\mu$ l

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(continued)

Compound	Volume
DNA sample (10 to 500 ng/ $\mu$ l)	1 $\mu$ l
H <sub>2</sub> O	qs 50 $\mu$ l

**[0053]** The following PCR program was used:

Temperature ( $^{\circ}$ C)	Time (min)	Number of cycles
94	2	1
94	1	35
50	1	
72	1	
72	5	1

**[0054]** The obtained PCR products are loaded on a 2.5% Agarose electrophoresis gel.

**[0055]** The figure 4 shows an electrophoresis gel of PCR products obtained with CF/CR primers realized on 2 OshV-1 isolates and 2 OshV-1  $\mu$ Var isolates. Only two genotype  $\mu$ Var (1a and 12) and two genotype reference OshV-1 (3 and 4) are presented. C: positive control. M: DNA ladder.

**[0056]** The results show that the  $\mu$ Var OshV-1 strain samples show a 158 pb amplicon, whereas the reference OshV-1 strain samples show a 173 pb amplicon enabling a simple and fast characterisation of OshV-1  $\mu$ Var strain (See fig 4).

## SEQUENCE LISTING

5 <110> IFREMER  
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SEGARRA, Amélie  
MORGA, Benjamin  
RENAULT, Tristan

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## Claims

1. A method for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, which method comprises the step of:
  - i) determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus, and
  - ii) determining if said OsHV-1 virus corresponds to the OsHV-1  $\mu$ Var strain.
2. The method of claim 1, wherein said determining step (ii) is done by identifying at least one nucleic acid polymorphism characteristic of the OsHV-1  $\mu$ Var strain selected in the group comprising:
  - For SEQ ID n°1, at least an A instead of a G at position 151, a C instead of AA at position 223, an insertion of GC at position 249 instead of no nucleic acid, a G instead of C at position 253, an A instead of G at position 257, no nucleic acid instead of AC at position 306, G instead of ACTACTACTACTGA (SEQ ID n°3) at position 360, A instead of G at position 482, and/or a A instead of G at position 488.
  - For SEQ ID n°2, no nucleic acid instead of an A at position 110 and/or a T instead of a C at position 526.
3. The method of any of claim 1 or 2, wherein said method comprises the step of determining the sequence of the C region (SEQ ID n°1) of the genomic DNA of said OsHV-1 virus.
4. The method of claim 3, wherein the determining step (ii) is done by identifying the polymorphism corresponding to G instead of ACTACTACTACTGA (SEQ ID n°3) at position 360 of the C region (SEQ ID n°1) of the genomic DNA of said OsHV-1 virus.
5. The method of any of claims 1 to 4, wherein said method is for diagnosing an infection by an OsHV-1  $\mu$ Var strain in a mollusc, wherein said method further comprises a previous step of determining the presence or the absence of an OsHV-1 virus in a biological sample of said mollusc, preferably said biological sample is a soft tissue of said mollusc.
6. The method of claim 5, wherein said mollusc is an oyster and preferably *Crassostrea gigas*
7. The method of any of claims 1 to 6, wherein the step (i) of determining the sequence of the C region (SEQ ID n°1) and/or of the IA region (SEQ ID n°2) of the genomic DNA of said OsHV-1 virus is done by nucleic acid amplification techniques and preferably by PCR.
8. The method of claim 7, wherein the determining step (i) is done by PCR using the pair of primers CR (GTG ATG GCT TTG GTC AAG GT, SEQ ID n°7) and CF (CCC CGG GGA AAA AGT ATA AA, SEQ ID n°8).
9. A pair of oligonucleotides useful for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, wherein said pair of oligonucleotides have the sequences GTG ATG GCT TTG GTC AAG GT (SEQ ID n°7) and (CCC CGG GGA AAA AGT ATA AA (SEQ ID n°8).
10. A kit for determining if a particular Ostreid herpesvirus 1 (OsHV-1) corresponds to the OsHV-1  $\mu$ Var strain, wherein said kit comprises the pair of oligonucleotides as defined in claim 10.

**Amended claims in accordance with Rule 137(2) EPC.**

5  
1. A method for determining if a particular Ostreid herpesvirus 1 (OshV-1) corresponds to the OshV-1  $\mu$ Var strain, which method comprises the step of:

- 10
- i) determining the sequence of the C region of the genomic DNA of said OshV-1 virus, wherein said C region has the sequence SEQ ID n°1 and
  - ii) determining if said OshV-1 virus corresponds to the OshV-1  $\mu$ Var strain by identifying the polymorphism corresponding to G instead of ACTACTACTACTGA (SEQ ID n°3) at position 360 of the C region of the genomic DNA of said OshV-1 virus.

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2. The method of claim 1, wherein said method is for diagnosing an infection by an OshV-1  $\mu$ Var strain in a mollusc, wherein said method further comprises a previous step of determining the presence or the absence of an OshV-1 virus in a biological sample of said mollusc.

3. The method of claim 2, wherein said biological sample is a soft tissue of said mollusc.

4. The method of claim 2, wherein said mollusc is an oyster.

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5. The method of claim 4, wherein said oyster is *Crossostrea gigas*.

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6. The method of any of claims 1 to 5, wherein the step (i) of determining the sequence of the C region of the genomic DNA of said OshV-1 virus is done directly by sequencing this region or indirectly by hybridization techniques, by enzymatic digestion techniques, by amplification techniques or by enzymatic techniques.

7. The method of claim 6, wherein the step (i) of determining the sequence of the C region of the genomic DNA of said OshV-1 virus is done by nucleic acid amplification techniques.

30  
8. The method of claim 7, wherein the step (i) of determining the sequence of the C region of the genomic DNA of said OshV-1 virus is done by PCR.

9. The method of any of claims 7 or 8, wherein the determining step (i) is done by PCR using the pair of primers:

- 35
- CR having the sequence GTG ATG GCT TTG GTC AAG GT (SEQ ID n°7), and
  - CF having the sequence CCC CGG GGA AAA AGT ATA AA (SEQ ID n°8).

40  
10. A pair of oligonucleotides useful for determining if a particular Ostreid herpesvirus 1 (OshV-1) corresponds to the OshV-1  $\mu$ Var strain, wherein said pair of oligonucleotides has the sequences:

- GTG ATG GCT TTG GTC AAG GT (SEQ ID n°7), and
- CCC CGG GGA AAA AGT ATA AA (SEQ ID n°8).

45  
11. A kit for determining if a particular Ostreid herpesvirus 1 (OshV-1) corresponds to the OshV-1  $\mu$ Var strain, wherein said kit comprises the pair of oligonucleotides as defined in claim 10.

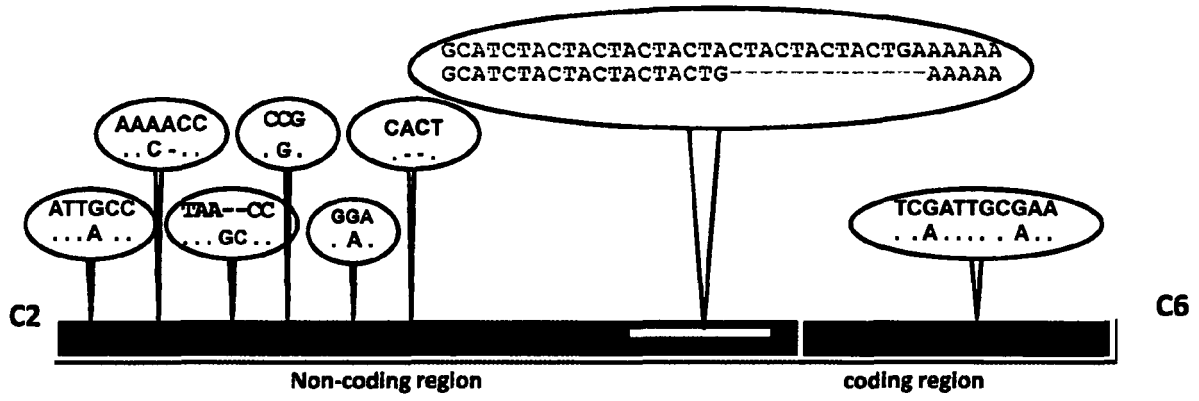


Figure 1

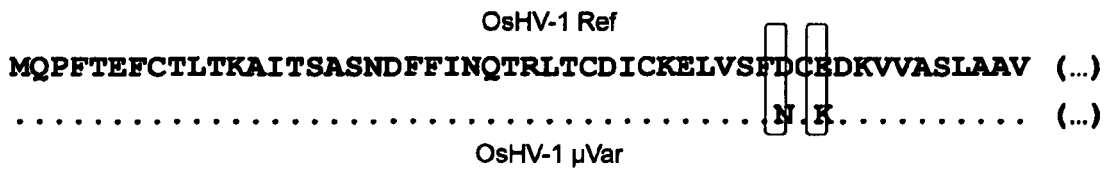


Figure 2

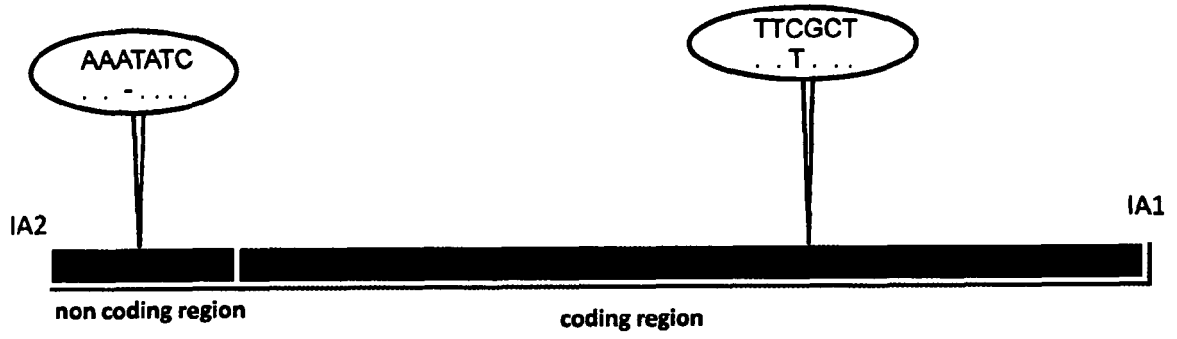


Figure 3

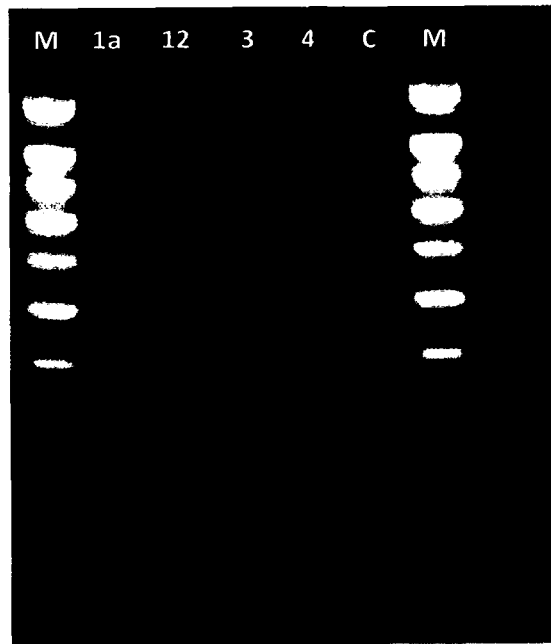


Figure 4



EUROPEAN SEARCH REPORT

Application Number  
EP 10 35 8001

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	PEPIN JEAN-FRANCOIS, SEGARRA AMELIE, FAURY NICOLE, SCHIKORSKI DAVID, COBRET LAËTITIA, RENAULT TRISTAN: "Summer mortality in 2008 and Ostreid Herpes virus investigations" INTERNET ARTICLE, [Online] 19 March 2009 (2009-03-19), XP002590667 Retrieved from the Internet: URL:http://en.scientificcommons.org/43814649> [retrieved on 2010-07-06]	1,3,5-7	INV. C12Q1/70
Y	* abstract *	2	
Y	----- ARZUL ISABELLE ET AL: "Evidence for interspecies transmission of oyster herpesvirus in marine bivalves" JOURNAL OF GENERAL VIROLOGY, vol. 82, no. 4, April 2001 (2001-04), pages 865-870, XP002590668 ISSN: 0022-1317 * abstract * * figure 1d *	2	
T	----- ANONYMOUS: "Implementing council directive 2006/88/EC as regards measures to control increased mortality in oysters of the species Crassostrea gigas in connection with the detection of ostreid herpes virus 1 microvar." INTERNET ARTICLE, [Online] 3 March 2010 (2010-03-03), XP002590669 Retrieved from the Internet: URL:http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:052:0001:0013:EN:PDF> [retrieved on 2010-07-06] * Annex I, Part B *		
			TECHNICAL FIELDS SEARCHED (IPC)
			C12Q
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
Munich		9 July 2010	Ripaud, Leslie
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

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EPO FORM 1503 03.82 (P04C01)



EUROPEAN SEARCH REPORT

Application Number  
EP 10 35 8001

DOCUMENTS CONSIDERED TO BE RELEVANT				
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)	
A	BATISTA ET AL: "Detection of ostreid herpesvirus 1 DNA by PCR in bivalve molluscs: A critical review" JOURNAL OF VIROLOGICAL METHODS, ELSEVIER BV, NL LNKD- DOI:10.1016/J.JVIROMET.2006.09.005, vol. 139, no. 1, 6 December 2006 (2006-12-06), pages 1-11, XP005794339 ISSN: 0166-0934 * abstract * * table 3 *	1-10		
A	----- COHENNEC-LAUREAU N, BAUD JP, BEDIER E, BOUDRY P, HUVET A, NICOLAS JL, PEPIN JF, PETTON B: "Bilan des "journées surmortalité des huitres creuses, Crassotrea gigas" du programme P7 "Aquaculture durable" des 8 et 9 décembre 2009" INTERNET ARTICLE, [Online] January 2010 (2010-01), XP002590670 Retrieved from the Internet: URL:http://archimer.ifremer.fr/doc/2010/ac te-7393.pdf> [retrieved on 2010-07-07] * page 2 *	1-10		TECHNICAL FIELDS SEARCHED (IPC)
A	----- US 2007/282001 A1 (CHI FRANCIS [HK]) 6 December 2007 (2007-12-06) * claim 10 * -----	1-10		
The present search report has been drawn up for all claims				
Place of search Munich		Date of completion of the search 9 July 2010	Examiner Ripaud, Leslie	
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document		

1  
EPO FORM 1503 03-82 (P04/C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 10 35 8001

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

09-07-2010

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007282001 A1	06-12-2007	AR 050596 A1	08-11-2006
		AT 409468 T	15-10-2008
		BR PI0512923 A	15-04-2008
		EP 1765317 A1	28-03-2007
		WO 2006002868 A1	12-01-2006
		JP 2008505140 T	21-02-2008
		KR 20070026873 A	08-03-2007
-----			

**REFERENCES CITED IN THE DESCRIPTION**

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**Non-patent literature cited in the description**

- **PEPIN.** *J VirolMethods*, 2008, vol. 149 (2), 269-76  
**[0027]**